

AMENDMENTS TO THE CLAIMS

This listing of claims replaces all prior versions, and listings, of claims in the present application.

1. (Currently Amended) An isolated nucleic acid comprising A
~~gene having a nucleotide sequence of a nucleic acid~~ selected from
the group consisting of:

(A) a nucleotide sequence ~~nucleic acid~~ encoding a polypeptide
having the amino acid sequence of SEQ ID NO: 14 ~~of the Sequence~~
~~Listing or a partial sequence thereof~~, wherein the polypeptide
possesses ~~possessing~~ a ceramidase activity;

(B) a nucleotide sequence comprising ~~nucleic acid having a~~
~~nucleotide sequence of~~ SEQ ID NO: 15, wherein said nucleotide
sequence encodes ~~of the Sequence Listing or a partial sequence~~
~~thereof and encoding~~ a polypeptide possessing a ceramidase
activity;

(C) ~~anucleic acid encoding a polypeptide consisting of an~~
~~amino acid sequence resulting from deletion, addition, insertion or~~
~~substitution of at least one amino acid residue in the amino acid~~
~~sequence of SEQ ID NO: 14 of the Sequence Listing, the polypeptide~~
~~possessing a ceramidase activity;~~

~~(D) a nucleic acid consisting of an amino acid sequence~~
~~resulting from deletion, addition, insertion or substitution of at~~

~~least one base in the nucleotide sequence of SEQ ID NO: 15 of the Sequence Listing and encoding a polypeptide possessing a ceramidase activity;~~

~~(E) a nucleotide sequence which hybridizes to the complement of the nucleotide sequence of (A) or (B) nucleic acid capable of hybridizing to a complementary strand of a nucleic acid of any one of the above (A) to (D), under stringent conditions of 6 x SSC containing 0.5% SDS, 5 x Denhardt's, and 100 µg/ml salmon sperm DNA at 50°C, and encoding wherein said nucleotide sequence encodes a~~
polypeptide possessing a ceramidase activity; and

~~(F) (D) a nucleotide sequence nucleic acid having a nucleotide sequence different from the nucleotide sequence nucleic acid of any one of the above (A) to (C) (E) via degeneracy of the genetic code, and encoding wherein said nucleotide sequence encodes a~~
polypeptide possessing a ceramidase activity.

2. (Currently Amended) The nucleic acid gene according to claim 1, wherein the ceramidase activity of the polypeptide is ~~capable of being~~ detected by the following steps:

(a) incubating an a-gene expression product in a reaction mixture comprising composition: 550 pmol of 12-((N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)dodecanoyl)sphingosine C12-NBD-ceramide and 1.0%(W/V) sodium cholate in 20 µl of 25 mM Tris-hydrochloric

acid buffer (pH 7.5)† at 37°C for 30 minutes, ~~to react the mixture;~~
and

(b) detecting generation of a 12-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)dodecanoyl acid ~~C12-NBD fatty acid~~ in the reaction product.

3. (Currently Amended) The nucleic acid gene according to claim 1 or 2, wherein the polypeptide exhibits at least the following characteristics:

(i) ~~action:~~ action of hydrolyzing ceramide, to generate sphingoid and a fatty acid;

(ii) substrate ~~specificity:~~ specificity of hydrolyzing N-acylsphingosine,† but not acting on galactosylceramide, sulfatide, GM1a, and sphingomyelin;

(iii) optimum ~~pH: being~~ pH of from 7.0 to 8.0; and

(iv) no lowering of the activity ~~not being found~~ when treated in 20 mM Tris-hydrochloric acid (pH 7.5) containing 0.1% polidocanol at 37°C for 24 hours, but ~~the activity~~ lowering of activity to about 30% by a treatment at 60°C for 1 hour ~~to about 30% of the activity before the treatment.~~

4. (Currently Amended) A recombinant DNA comprising the gene nucleic acid of claim 1.

5. (Currently Amended) An expression vector ~~for a microorganism, an animal cell or a plant cell,~~ comprising the gene nucleic acid of claim 1 or the recombinant DNA of claim 4.

6. (Currently Amended) A transformant ~~carrying~~ comprising the expression vector of claim 5.

7. (Currently Amended) A method for producing a polypeptide possessing a ceramidase activity, ~~characterized by~~ comprising the steps of

culturing the transformant of claim 6 under conditions appropriate for expression ~~of the ceramidase gene and production of the polypeptide encoded by the gene,~~ and

collecting a polypeptide possessing a ceramidase activity from the resulting culture.

8. (Currently Amended) An isolated A polypeptide ~~having the comprising the~~ amino acid sequence of SEQ ID NO: 14, wherein said polypeptide possesses ~~of the Sequence Listing or a partial sequence thereof and possessing~~ a ceramidase activity.

9. (Currently Amended) An isolated A polypeptide possessing a ceramidase activity, wherein said polypeptide is encoded by the gene nucleic acid of claim 1.

10. (Currently Amended) The polypeptide according to claim 8 or 9, wherein the ceramidase activity is ~~capable of being~~ detected by the following steps:

(a) incubating an a-gene expression product in a reaction mixture comprising ~~composition~~ 550 pmol of 12-((N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)dodecanoyl)sphingosine C12-NBD-ceramide and 1.0%(W/V) sodium cholate in 20 μ l of 25 mM Tris-hydrochloric acid buffer (pH 7.5) at 37°C for 30 minutes, ~~to react the mixture;~~ and

(b) detecting generation of a 12-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)dodecanoyl acid ~~C12-NBD fatty acid~~ in the reaction product.

11. (Currently Amended) An isolated antisense DNA which is complementary to the gene nucleic acid of claim 1 ~~or a part thereof.~~

12. (Amended) An isolated antisense ~~Antisense~~ RNA which is complementary to the ~~gene~~ nucleic acid of claim 1 ~~or a part thereof~~.

13. (Original) An expression vector comprising the antisense DNA of claim 11.

14. (Amended) An isolated oligonucleotide probe or primer which, ~~capable of specifically~~ hybridizes under stringent conditions of 6 x SSC, containing 0.5% SDS, at a temperature of ((Tm of the oligonucleotide)-25°C) ~~hybridizing~~ to the ~~gene~~ nucleic acid of claim 1, or a complementary stand thereof, wherein said oligonucleotide probe or primer has a strand length of 15 to 40 bases.

15. (Withdrawn) An antibody or a fragment thereof, capable of specifically binding to the polypeptide of claim 8.

16. (Currently Amended) A method for detecting a nucleotide sequence ~~gene~~ encoding a polypeptide possessing a ceramidase activity, comprising using the oligonucleotide probe or primer of claim 14.

17. (Currently Amended) A kit for the use in detection of a nucleotide sequence ~~gene~~ encoding a polypeptide possessing a ceramidase activity, comprising the oligonucleotide probe and/or primer of claim 14.

18. (Withdrawn) A method for detecting a polypeptide possessing a ceramidase activity, comprising using the antibody or a fragment thereof of claim 15.

19. (Withdrawn) A kit for use in detection of a polypeptide possessing a ceramidase activity, comprising the antibody or a fragment thereof of claim 15.

20. (Currently Amended) A method of controlling an amount of ceramide in a cell and/or in a tissue, comprising the step of ~~characterized by~~ introducing the nucleic acid ~~gene~~ of claim 1 or an antisense nucleic acid thereof into the cell and/or the tissue, thereby controlling the amount of ceramide in the cell and/or in the tissue.

21. (New) The oligonucleotide probe or primer according to claim 14, wherein said oligonucleotide probe or primer is derived from a region having a sequence characteristic of a ceramidase.